Sensitization to Hymenoptera venoms is common, but systemic sting reactions are rare

Gunter J. Sturm, MD, PhD,[a] Bettina Kranzelbinder, MD,[a] Christian Schuster, MD,[a] Eva M. Sturm, PhD,[b] Danijela Bokanovic, MD,[a] Jutta Vollmann, Msc,[c] Karl Crailsheim, PhD,[c] Wolfgang Hemmer, PhD,[d] and Werner Aberer, MD[a] *Graz and Vienna, Austria*

Background: Sensitization to Hymenoptera venom without systemic sting reactions (SSRs) is commonly observed in the general population. Clinical relevance for a future sting has not yet been investigated.

Objective: We aimed to evaluate the effect of these debatable sensitizations with deliberate sting challenges and to monitor serologic changes for up to 2 years.

Methods: One hundred thirty-one challenges with bees and wasps were performed in 94 subjects with a hitherto irrelevant sensitization. The clinical outcome was recorded, and results of specific IgE (sIgE) determinations, skin tests, and basophil activation tests were correlated to the sting reaction. sIgE levels were monitored in reactors and nonreactors after 3 hours, 1 week, 4 weeks, and 1 year.

Results: Only 5 (5.3%) patients had SSRs, but 41 (43.6%) had large local reactions (LLRs) after the sting. Compared with the general population, there was a 9.5-fold higher risk for LLRs but not for SSRs. Three hours after the sting, sIgE levels slightly decreased, but none of the 94 subjects' results turned negative. After 1 week, sIgE levels already increased, increasing up to 3.5-fold (range, 0.2- to 34.0-fold) baseline levels after 4 weeks. To assess the clinical relevance of this increase, we randomly selected 18 patients for a re-sting. Again, 50% had an LLR, but none had an SSR.

Conclusion: Although sensitization to Hymenoptera venoms was common, the risk of SSRs in sensitized subjects was low in our study. The sIgE level increase after the sting was not an indicator for conversion into symptomatic sensitization. Currently available tests were not able to distinguish between asymptomatic sensitization, LLRs, and SSRs. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: *Asymptomatic sensitization, basophil activation test, component-resolved diagnosis, IgE determination, intradermal test, large local reaction, sting challenge, systemic sting reaction, total IgE*

Abbreviations used
AS: Asymptomatic sensitization
BAT: Basophil activation test
CCD: Cross-reactive carbohydrate determinant
CRD: Component-resolved diagnosis
IDT: Intradermal test
LLR: Large local reaction
sIgE: Specific IgE
SSR: Systemic sting reaction
tIgE: Total IgE

Depending on the climate, the prevalence of Hymenoptera stings ranges from 56.6% to 94.5% in the general adult population.[1] In the general population 0.3% to 7.5% are reported to have experienced systemic sting reactions (SSRs), and 2.4% to 26.4% have had large local reactions (LLRs) to Hymenoptera stings.[2] Recently, we carried out the first epidemiologic telephone survey on Hymenoptera venom allergy in Austria and found that 3.3% have experienced SSRs and 4.6% have experienced LLRs, respectively.[3]

Asymptomatic sensitization (AS) to bee and wasp venom occurs frequently in *in vitro* tests, and 27.1% to 40.7% of the general population are reported to have detectable specific IgE (sIgE) to Hymenoptera venoms.[4,5] Furthermore, AS is related to total IgE (tIgE) levels; in healthy subjects with high tIgE levels, sIgE to Hymenoptera venoms was demonstrable in up to 66.7% of investigated subjects.[5] Therefore current criteria to diagnose Hymenoptera venom allergy cannot accurately predict the occurrence or severity of anaphylactic symptoms after a sting. The main cause of AS in subjects double sensitized to bee and wasp venom is the presence of sIgE to cross-reactive carbohydrate determinants (CCDs) in the serum.[6,7] Many of the bee venom allergens and some of the wasp venom allergens bear CCDs; therefore sensitization to CCDs can mimic double sensitization to Hymenoptera venoms.[7] However, sIgE to CCDs as a cause for AS in monosensitized subjects is typically not observed.[5]

Nevertheless, a large portion of subjects are sensitized to nonglycosylated venom allergens and tolerate Hymenoptera stings well. Given that the prevalence of SSRs in Austria is 3.3% and that 40.7% of the general population are sensitized to at least 1 venom, it can be assumed that the majority of sensitized subjects will not experience SSRs. However, sensitization could have been converted into clinically relevant hypersensitivity after the recent sting, and therefore subjects could potentially react to the next sting. The relevance of these unclear sensitizations has not been elucidated. Therefore we aimed to conduct a prospective study to clarify the effect of detectable sIgE to Hymenoptera venoms for the next sting. For this purpose, deliberate sting

From [a]the Department of Dermatology, Division of Environmental Dermatology and Venerology, and [b]the Institute of Experimental and Clinical Pharmacology, Medical University of Graz; [c]the Institute of Zoology, University of Graz; and [d]Floridsdorf Allergy Center, Vienna.

Supported by a grant from the Austrian Society for Dermatology and Venereology.

Disclosure of potential conflict of interest: G. J. Sturm has received research support from OEGDV and has received lecture fees from ALK-Abelló. C. Schuster has received lecture fees from the Austrian Workers' Compensation Board and ALK-Abelló. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication August 7, 2013; revised October 24, 2013; accepted for publication October 25, 2013.

Corresponding author: Gunter J. Sturm, MD, PhD, Department of Dermatology, Medical University of Graz, Auenbruggerplatz 8, A-8036 Graz, Austria. E-mail: gunter.sturm@medunigraz.at.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2013.10.046

challenges with living bees and wasps were performed. Several years ago, we found that patients with low tIgE levels (<50 kU/L) had a higher risk for severe reactions.[8] We also observed that high tIgE levels (>250 kU/L) were associated with a higher frequency of AS.[5] Therefore we initially aimed to evaluate whether subjects with low tIgE levels were predisposed to (severe) SSRs or LLRs. Then we correlated test results of available diagnostic tools, such as the skin prick test, the intradermal test (IDT), sIgE determination, component-resolved diagnosis (CRD), and the basophil activation test (BAT) with the outcome of the sting challenge. We also monitored sIgE levels against the respective venom after sting challenges. In this context we tested the hypothesis of sIgE "consumption" because of exposure to venom allergens after a sting. Because of increased sIgE levels after the first challenge, we performed a second sting challenge in randomly selected subjects to investigate whether this increase was associated with the conversion of a clinically irrelevant sensitization into relevant hypersensitivity.

METHODS

Subjects

Subjects who tolerated previous Hymenoptera field stings without an SSR were initially screened for sIgE to Hymenoptera venom. Subjects with detectable sIgE to at least 1 Hymenoptera venom who met all other inclusion and exclusion criteria (see Table E1 in this article's Online Repository at www.jacionline.org) were asked to participate in the study. Finally, 110 subjects were enrolled. After a complete health check, including physical examinations, laboratory tests, spirometry, and electrocardiography, diagnostic tests for Hymenoptera venom allergy were performed. An atopic disposition was considered if there was at least 1 positive reaction to an allergen in a skin prick test panel of common aeroallergens. Forty-one were stung by a bee, 16 by a wasp, and 37 by both a bee and a wasp. In subjects with double sensitization, 2 sting challenges were performed on the same day. If the first sting was tolerated, another challenge was performed after 3 hours. Sting challenges were followed by repeated serologic tests (Fig 1). This study was approved by the ethics committee of the Medical University of Graz (approval no. 18-046).

Classification of sting reactions

According to the modified classification of Ring and Messmer,[2] generalized skin symptoms, such as flush, urticaria, and angioedema, were classified as grade I reactions. Mild-to-moderate respiratory, cardiovascular, or gastrointestinal symptoms were rated as grade II reactions. Bronchoconstriction, emesis, anaphylactic shock, and loss of consciousness were classified as grade III reactions. An LLR was defined as swelling exceeding a diameter of 10 cm that lasts longer than 24 hours.

Skin tests

The nature of sensitization was confirmed by means of standardized end point titration skin prick tests (10, 100, and 300 μg/mL) and IDTs (0.02 mL of 0.01, 0.1, and 1 μg/mL) with purified honeybee and vespid venom extracts (ALK-Abelló, Hørsholm, Denmark). Skin prick test and IDT results were considered positive in the presence of wheals 3 and 5 mm larger in diameter and erythema, respectively.

Determination of sIgE and tIgE levels

Specific and tIgE antibody levels in the patients' sera were measured by using ImmunoCAP 1000 (Thermo Fisher Scientific, Waltham, Mass), Immulite 2000 (Siemens, Tarrytown, NY), and ADVIA Centaur (Siemens), according to the manufacturer's instructions. CRD with rApi m 1 and rVes v 1 and 5 was done on the ImmunoCAP 1000. Diagnosis with nApi m 1, nVes v 1, and nVes v 5 was done on the ADVIA Centaur platform at the Department of I+D (ALK-Abelló, Madrid, Spain). For some analyses, patients were grouped according to low (<50 kU/L), intermediate (50-250 kU/L), and high (>250 kU/L) tIgE levels.

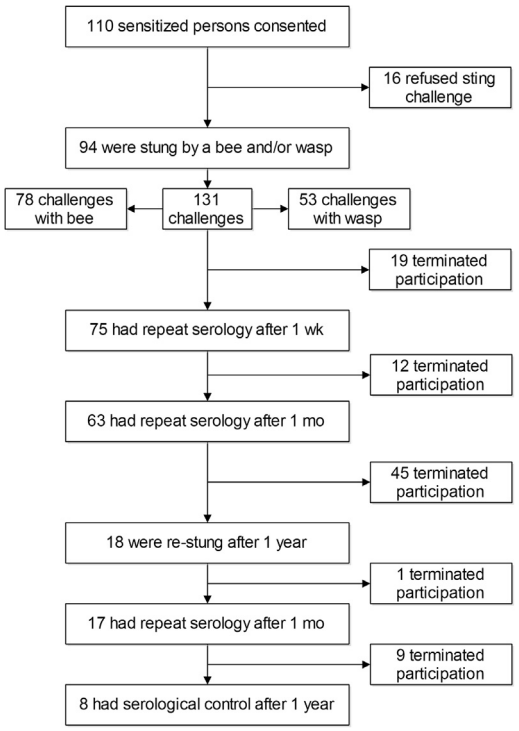

FIG 1. Screening, sting challenges, and follow-up. Initially, 110 sensitized subjects consented to participate in the study. Control visits were optional, and therefore some subjects discontinued the study prematurely.

BAT

BATs were performed, as previously described.[9,10] In brief, cell samples were analyzed by using 3-color flow cytometry (FC 500; Beckman Coulter, Fullerton, Calif). Basophils were identified as a single population of cells that stained positively for CD123 (FL-2) and negatively for HLA-DR (FL-4). Upregulation of CD63 expression was indicated by an increase in fluorescence in the FL-1 channel. Acquisition was terminated after 500 basophil target events. A 2.5-fold increase in the number of activated basophils (>25%) compared with the negative control (10%) at any of the test concentrations of the allergen was considered a positive response.

Deliberate sting challenge test

The insect sting challenge test was carried out with a definitely identified living bee (*Apis mellifera*) and/or wasp (*Vespula germanica* and *Vespula vulgaris*) supplied by the Institute of Zoology, Graz, Austria. The choice of the insect or insects was based on detectable sIgE with the CAP system. The challenge was performed on the upper forearm under partial inpatient conditions with intensive medical stand-by and a continuous infusion; it was considered valid if a wheal of 5 mm or greater in diameter and erythema after 15 minutes at the site of the sting occurred.

Data analysis

All data are expressed as medians (25% to 75% percentiles) on the raw scale, unless otherwise indicated. Data were tested for normality by using the Kolmogorov-Smirnov test. Continuous variables were analyzed by using the Kruskal-Wallis test; categorical variables were compared by using χ^2 or Fisher exact tests. The Cohen κ coefficient was calculated to check agreement

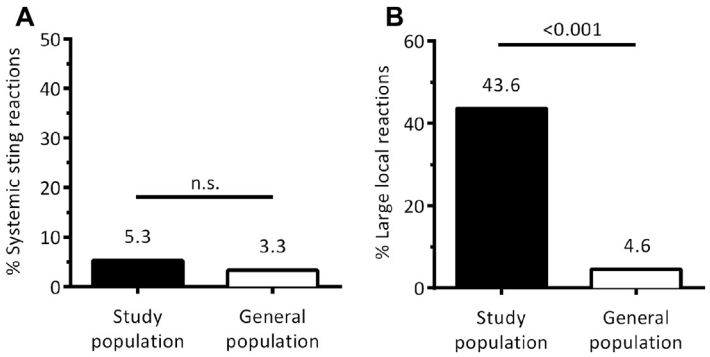

FIG 2. Frequency of SSRs and LLRs compared with the general population. **A,** Although the frequency of SSRs was slightly higher in the study population, the difference to the general population was not statistically significant. **B,** Notably, the risk of LLRs was approximately 9.5-fold higher compared with the general population. Data of the general population were obtained by means of telephone survey of accidental stings.[3] The study population was sensitized subjects undergoing deliberate sting challenges. *n.s.,* Not significant.

between the tests. The level of significance was set at a P value of less than .05. IBM SPSS Statistics 20.0 (IBM, Somers, NY) was used for statistical analysis.

RESULTS

History and demographic data

Sixteen of 110 subjects refused sting challenges. Of 94 included subjects, 44 (46.8%) were male, and 50 (53.2%) were female. Median age was 29.0 (25% to 75% percentile, 24.0-40.3 years). Forty-eight (51.1%) were considered to have an atopic predisposition, and 27 (28.7%) reported previous LLRs caused by Hymenoptera stings.

Outcome of sting challenges

Finally, 131 sting challenges with bees, wasps, or both were performed in 94 subjects. Forty-one were stung by a bee, 16 by a wasp, and 37 by both a bee and a wasp. Only 6 SSRs in 5 (5.3%) of 94 subjects were observed. However, LLRs were frequently observed: 41 (43.6%) of 94 had LLRs within 24 hours after the sting; 16 (39.0%) of them reported LLRs previously. Compared with the general population, there was a 9.5-fold higher risk for LLRs but no increased risk for SSRs (Fig 2).[3] According to the classification of Ring and Messmer, 4 grade I reactions, 1 grade II reaction, and 1 grade III reaction were observed (Table I). Subjects with previous LLRs did not appear to have a higher risk for SSRs: 3 (4.5%) of 64 without a history of an LLR had an SSR, and 2 (7.4%) of 25 who have previously experienced LLRs had SSRs ($P = .623$).

Association of SSRs with tIgE levels

On the basis of our experience, we would have expected more SSRs and, particularly, a higher frequency of SSRs in subjects with lower tIgE levels.[8] One (2.7%) of 37 subjects with low IgE levels experienced an SSR, and 1 (3.4%) of 29 with intermediate levels and 3 (10.7%) of 28 with high tIgE levels were reactors. There were even more SSRs in subjects with high tIgE levels. However, differences were not significant ($P = .384$), but the unexpected low number of reactions might invalidate the statistics. Similar observations were made with tIgE levels and LLRs: the occurrence of LLRs was not associated with low or high tIgE levels ($P = .139$).

TABLE I. Clinical outcomes in subjects with SSRs after sting challenges

Subject	Insect	Onset (min)	Symptoms	Grade
4	Bee	25	Flush, urticaria	1
19	Bee	5	Tachycardia, mild decrease in blood pressure	2
26	Bee	20	Isolated wheals	1
26	Wasp	25	Mild periorbital edema	1
41	Wasp	10	Tachycardia, vomiting, defecation	3
69	Wasp	20	Isolated wheals	1

Six SSRs in 5 subjects were observed. One subject had SSRs to both bee and wasp stings.

Outcome of sting challenges compared with test results

Results of all tests (sIgE determination with the CAP, Immulite, and ADVIA; CRD, skin prick tests, and IDTs) except the BAT were positive in all subjects reacting with SSRs to wasp stings. The situation was different in subjects who had SSRs after bee stings: 2 of 3 subjects were unable to receive diagnoses based on rApi m 1 values (CAP) and the BAT (Table II).

In a next step the frequency of positive test results in subjects with negative sting challenge results was determined. Positive test results, even to venom components, were commonly observed but were irrelevant with respect to SSRs (Fig 3). We further sought to explore whether sensitization was associated with the occurrence of LLRs. LLRs were less frequently observed after bee stings: 26 (33.3%) of 78 subjects stung by a bee and 29 (54.7%) of 53 subjects challenged by a wasp sting had LLRs ($P = .019$). We aimed to check which test correlated best with the occurrence of LLRs. We also hypothesized that a genuine sensitization confirmed by means of CRD might be associated with LLRs. However, among the performed tests, only positive BAT results appeared to be more frequent in subjects with LLRs. Surprisingly, even sIgE levels to venom components were frequently positive in subjects without SSRs and LLRs (Fig 4). The low frequency of positive test results for rApi m 1 was primarily caused by the low sensitization rate of 57% to 82%[11-14] to Api m 1 and was not a matter of good specificity.

Neither using different thresholds for positivity of 0.36, 0.7, 1.0, or 1.7 kU/L for sIgE to allergen components nor

TABLE II. Test results in subjects with SSRs after sting challenge

Subject	CAP bee (kU/L)	Immulite bee (kU/L)	ADVIA bee (kU/L)	CAP rApi m 1 (kU/L)	ADVIA nApi m 1 (kU/L)	Skin prick test	IDT	BAT
4	2.92	5.60	2.27	0.38	3.49	10	0.01	1.79
19	0.64	1.73	1.21	0.28	1.66	300	0.1	40.00
26	1.47	3.26	0.71	0.04	0.71	100	1	14.00
Positive	3/3	3/3	3/3	1/3	3/3	3/3	3/3	1/3
Subject	**CAP wasp**	**Immulite wasp**	**ADVIA wasp**	**CAP rVes v 1/5**	**ADVIA nVes v 1/5**	**Skin prick test**	**IDT**	**BAT**
26	1.98	8.44	10.30	0.70/0.02	1.56/0.15	300	0.1	17.00
41	1.23	1.89	1.68	0.08/3.73	0.05/3.05	10	0.1	28.13
69	0.44	0.83	0.98	0.04/1.19	0.02/1.00	100	0.1	26.47
Positive	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3

There was a clear weakness of rApi m 1 (CAP) and BAT to diagnose bee venom allergy. In contrast to subjects allergic to bee venom, results of all tests except the BAT were positive in subjects with wasp venom allergy.
Skin prick test, Positive at micrograms; *IDT*, positive at micrograms; *BAT*, maximal basophil activation percentage; *positive*, greater than 25% activated basophils.

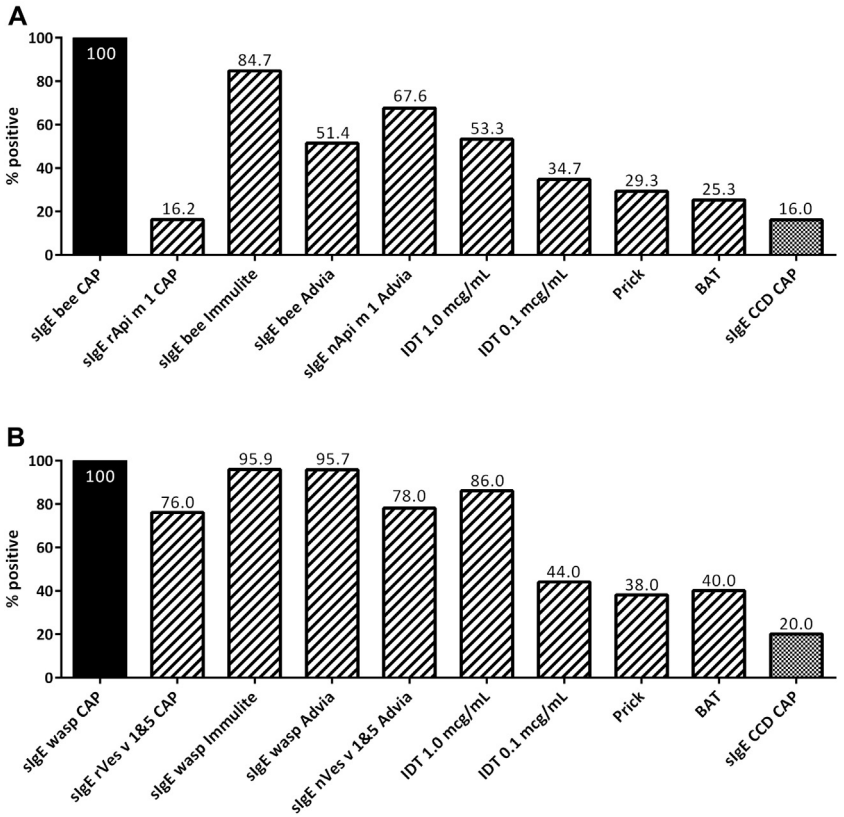

FIG 3. Outcome of tests in subjects who tolerated sting challenges without SSRs. **A,** Positive test results in subjects who were stung by a bee. Skin tests and the BAT showed a lower rate of positive results. Because of the known low sensitivity of rApi m 1, the good correlation of rApi m 1 with the sting challenge should be viewed critically. **B,** Positive test results in subjects who were stung by a wasp. Importantly, even sIgE to the major wasp venom components was detectable in the majority of subjects.

differentiation between CCD-positive and CCD-negative subjects allowed discrimination between asymptomatic subjects and subjects with LLRs. Interestingly, only 11 (12.4%) of the 89 nonreactors had detectable sIgE against CCDs. On the other hand, one of the 5 reactors also had sIgE against CCDs.

Time course of sIgE after the sting challenge

Three hours. First, we tested whether sIgE was "consumed" after a Hymenoptera sting. There was only a slight decrease in sIgE levels 3 hours after a wasp or bee sting: −0.08 kU/L for wasp venom, −0.13 for bee venom, −0.01 for Api m 1, and −0.10 for Ves v 5 (Fig 5, *A-D*). However, this decrease was highly statistically significant for all tests but Api m 1 ($P = .001$). Although there was only a minimal change in median values, the Wilcoxon test revealed that sIgE levels decreased in 55 (78.5%) of 70 bee venom–sensitized subjects and in 36 (76.6%) of 47 subjects with wasp venom sensitization. Of those who initially had sIgE to Ves v 5, 20 (90.9%) of 22 showed a decrease in sIgE levels to Ves v 5 after the sting. The situation in reactors was similar: 2 showed slightly increased and 4 showed slightly decreased sIgE levels after the sting (Fig 5, *E*). However, neither in

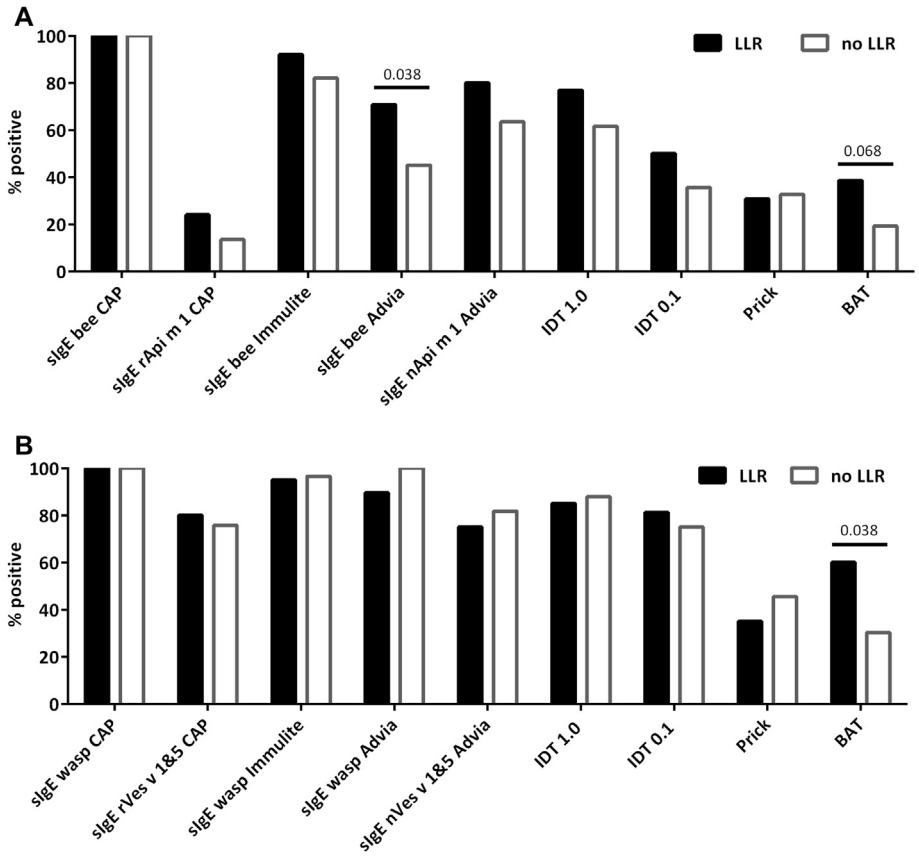

FIG 4. Outcome of tests in subjects with and without LLRs. **A,** Positive test results in subjects who were stung by a bee. Unexpectedly, the frequency of positive test results in subjects with LLRs was mostly similar to that seen in those with no LLRs. Agreement of all test results with LLRs was weak ($\kappa < 0.35$). **B,** Positive test results in subjects who were stung by a wasp. Contrary to subjects having had bee stings, there was no difference in the sensitization rate between the tests. Only the BAT showed more positive results in subjects with LLRs. Similar to bee sensitizations, agreement of all test results with LLRs was weak ($\kappa < 0.35$).

asymptomatic sensitized subjects nor in reactors did the initially positive sIgE result turn negative.

One to 4 weeks. sIgE levels increased markedly 1 week after the sting: there was a 2.2-fold increase in wasp venom sIgE levels and a 2.7-fold increase in sIgE levels to bee venom. After 4 weeks, the increase was 2.7-fold in sIgE levels to wasp venom and 5.0-fold in sIgE levels to bee venom. However, increases of up to 34-fold were recorded. Weaker but also significant increases in sIgE levels to the main allergens Api m 1 and Ves v 5 were also observed (Fig 5, *A-D*). Because control visits were optional, we could only analyze the complete sIgE time course of 3 reactors. Although data are limited, the increase in sIgE levels appeared to be similar; increases of up to 40-fold (subject 69) were observed (Fig 5, *E*).

Second sting challenge

The clear increase in sIgE levels prompted us to assess the clinical relevance for a second time in 18 randomly selected patients 1 year after the first challenge. In total, 19 sting challenges were performed. Sixteen were challenged with a bee ting, 1 with a wasp sting, and 1 with both a bee and a wasp sting. Because of the low number of wasp sting challenges, data of bee and wasp stings were analyzed together. Interestingly, there were more positive IDT and BAT results compared with the first challenge, whereas the number of positive results with CRD was the same as 1 year before (Fig 6, *A*).

Nine of 18 challenged subjects had an LLR, and therefore there was no significant change in the relative frequency of LLRs (Fig 6, *B*). Again, agreement of test results with the occurrence of LLRs was weak, even though the IDT result was more often positive in subjects with LLRs (Fig 6, *C*).

Time course of sIgE after the first and second sting challenges

The first sting caused significant increases in sIgE levels to the major allergens and to bee and wasp venom extracts. After 1 year, increased sIgE levels have decreased almost to baseline. Therefore baseline values before the first and second stings did not differ significantly. After the second challenge, sIgE levels of the 17 subjects stung by a bee increased again after 4 weeks to levels comparable with those after the first sting. However, the relative increase was more moderate (3.3-fold) because of the slightly increased baseline before the second sting. Therefore only a statistical trend could be calculated for this observation (Fig 7, *A*).

Then, to minimize the effect of individually differing baselines for sIgE to bee and wasp venom and to better visualize the kinetics

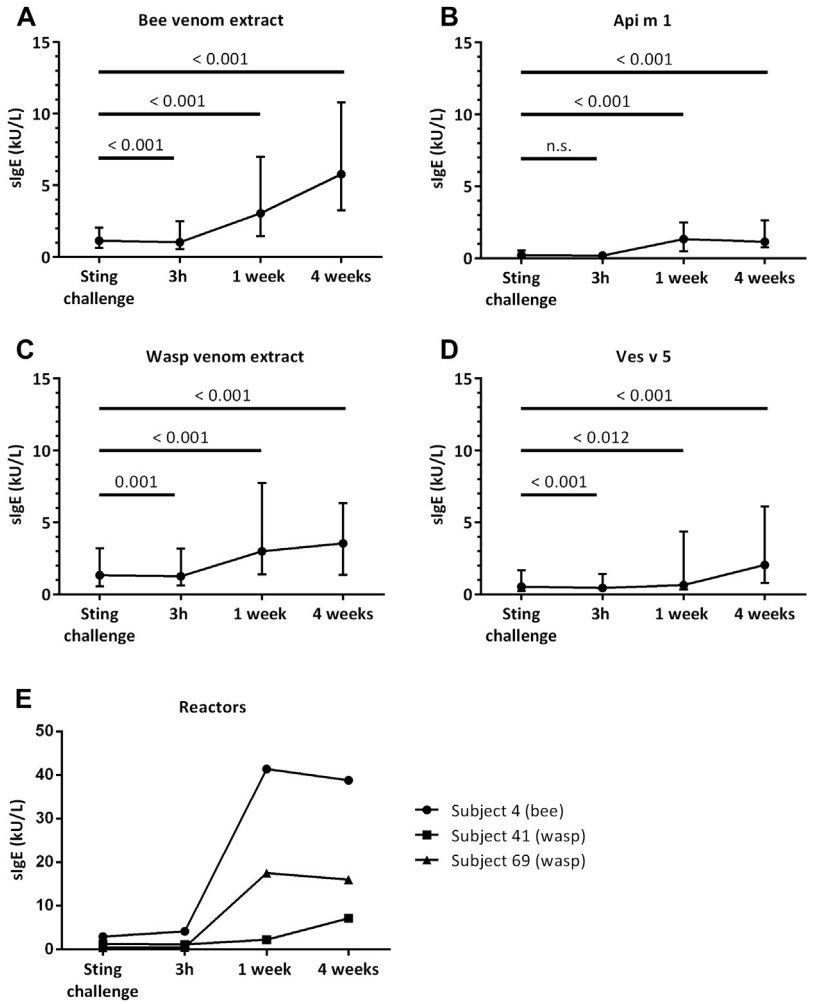

FIG 5. Time course of sIgE levels after the sting. Generally, there was a slight but significant decrease in sIgE levels 3 hours after the sting in asymptomatic sensitized subjects **(A, C, D)**. Only sIgE levels to Api m 1 remained stable **(B)**. Of the reactors **(E)**, 2 subjects showed a slight increase and 4 showed a slight decrease in sIgE levels (subjects 19 and 26 because of missing data for week 1 and 4 subjects not shown in Fig 5, *E*). In both nonreactors and reactors, sIgE levels markedly increased after only 1 week. Fig 5, *A*, n = 46 to 75; Fig 5, *B*, n = 26 to 28; Fig 5, *C*, n = 33 to 50; Fig 5, *D*, n = 22 to 24. Fig 5, *E*, Complete sIgE time course of 3 reactors. *n.s.*, Not significant.

of sIgE, values were calculated as x-fold increase of the baseline. As shown in Fig 7, *B*, the 2.1-fold increase in sIgE levels to bee and wasp venom extracts did not reach statistical significance. Only the 2.6-fold increase in sIgE levels to the major bee and wasp allergen was significant (Fig 7, *C*). Again, 1 year after the second sting, sIgE levels consistently decreased to baseline levels.

DISCUSSION

Positive test results to Hymenoptera venom despite a negative history are a well-known phenomenon in the general population. In older studies with IgE determination on the RAST platform, 27.1% were reported to have sIgE to Hymenoptera venoms,[4] and using the refined fluorescence enzyme assay (ImmunoCAP), 40.7% had detectable sIgE to any Hymenoptera venom.[5] In the initial phase of this study, 44.1% of all screened subjects had positive results. This rate of sensitization is quite high and cannot be explained by unspecific sensitization caused by CCDs because only 12.4% of the nonreactors had sIgE to CCDs. However, the actual number of patients with sIgE to CCDs might be underestimated given that the sensitivity of CCD screening tests with the ImmunoCAP platform is low.[6] Nevertheless, 76% of subjects with a sensitization to vespid venom were positive to the CCD-free venom components rVes v 1 and 5. In patients with bee venom hypersensitivity, the low sensitization rate to rApi m 1 was repeatedly described.[12,13] Therefore the low frequency of rApi m 1–positive subjects (CAP platform) is not surprising and not a matter of good specificity. The situation is different with nApi m 1 on the ADVIA platform. Sensitivity has been described to be higher compared with that of rApi m 1,[15] and therefore AS is more frequent as well. The reasons for the better sensitivity of nApi m 1 are a debated issue, and there might be a difference in the structure between the recombinant and natural forms. However, it bears CCDs, which is a problem for specificity in patients with increased tIgE levels.

Despite detectable sIgE, only 5.3% had SSRs after the sting challenge. Subjects with previous LLRs did not appear to be at a higher risk of SSRs. However, statistical analysis was limited by

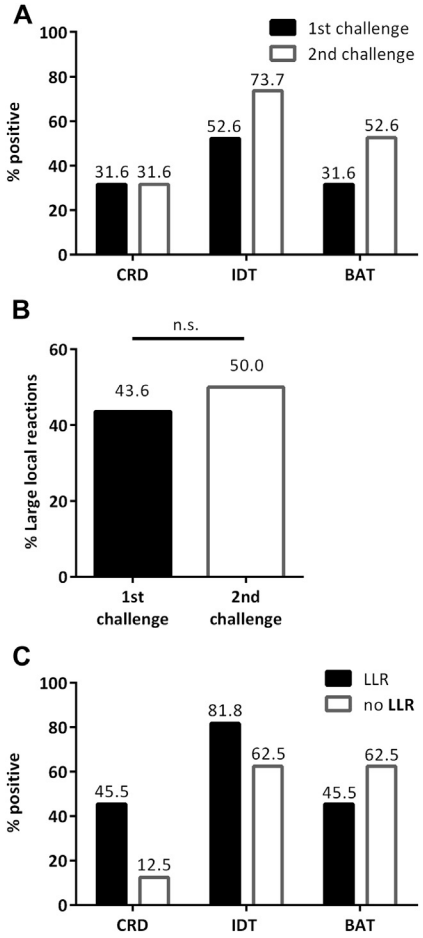

FIG 6. Outcome of repetitive sting challenges and correlation with test results. **A,** One year after the first sting challenge, more positive IDT and BAT results were observed. However, all repetitive sting challenges were tolerated without SSRs. **B,** The first sting challenge did not induce a significant increase in LLRs. **C,** Although CRD and IDTs revealed more positive results in subjects with LLRs, there was only a weak agreement between tests and the occurrence of LLRs (κ for all tests <0.35).

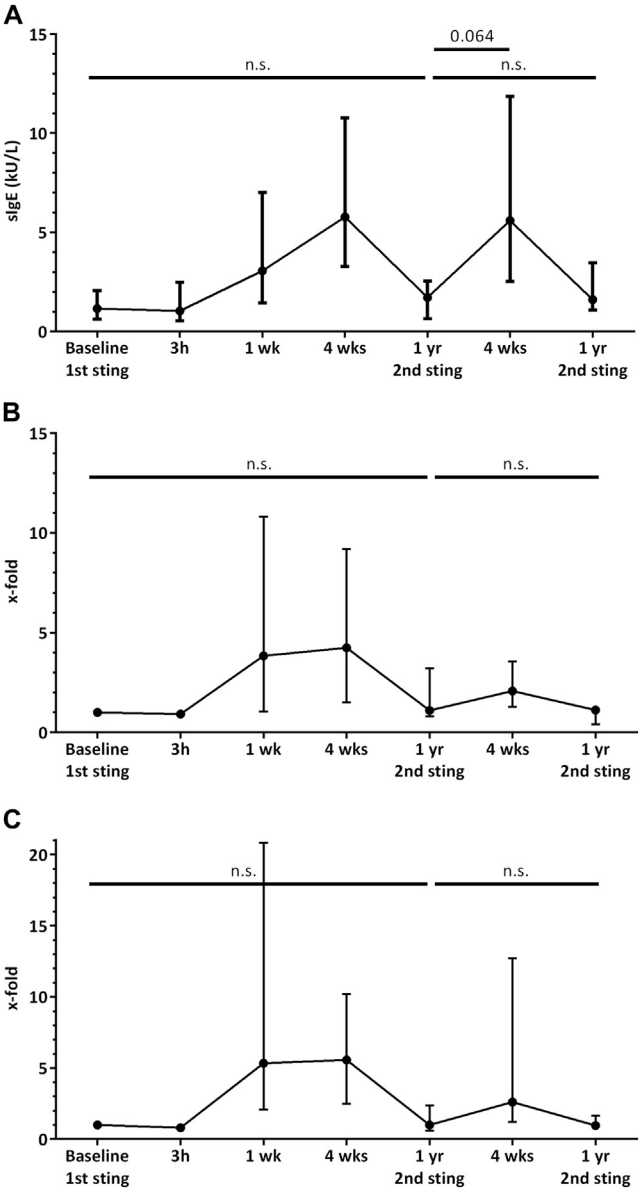

FIG 7. Time course of sIgE over a period of 2 years. **A,** sIgE levels to bee venom extract. After an initial marginal decrease directly after the first sting, sIgE levels increased to 5-fold of baseline. After 1 year, levels had decreased near baseline, increasing and decreasing again after the second challenge (n = 10-17). **B,** Normalized sIgE levels to bee and wasp venom extracts. Values are expressed as x-fold increase of baseline. Kinetics of sIgE were similar to those of quantitative levels. However, the increase in sIgE levels was only 2.1-fold after the second challenge compared with the 3.8-fold increase 1 week and 4.2-fold increase 4 weeks after the first challenge. (n = 10-19). **C,** Normalized levels of the major allergens Api m 1 and Ves v 5. The increase in sIgE levels to major allergens was slightly more pronounced compared with sIgE levels to the venom extracts. sIgE levels increased to 5.3-fold after 1 week and 5.6-fold after 4 weeks. After the second challenge, a modest increase to 2.6-fold was observed (n = 10-19). *n.s.*, Not significant.

the low number of SSRs. The frequency of reactors is comparable with the risk of the general population and far less than the risk for a re-sting reaction in allergic patients, which was reported between 25% to 52% after deliberate sting challenges.[16-18] By specifying the categorical concentration of tIgE, we recently observed that the frequency of AS was associated with tIgE levels: 23.1% of subjects with a tIgE level of less than 50 kU/L were sensitized to Hymenoptera venoms, whereas 66.7% with tIgE levels of greater than 250 kU/L had positive results.[5] Therefore we initially hypothesized that subjects with low tIgE levels are more likely to have an unknown clinically relevant hypersensitivity. However, this was not the case in this study; there were even more SSRs in subjects with high tIgE levels. Again, the low number of reactors hampered statistical analysis. A considerable proportion of subjects had LLRs; the risk for an LLR was about 10 times higher than in the general population. This supports the hypothesis that most of those reactions are IgE-mediated late-phase reactions.[19] Taken together, 51.1% of sensitized subjects tolerated stings well and experienced neither SSRs nor LLRs because of the sting. From the methodological point of view, the relatively high amount of venom that is usually used for *in vitro* diagnosis might be an issue. The high amount is essential to minimize competition between the low quantity of IgE and the substantial quantity of IgG. Therefore low-affinity cross-reacting sIgE with questionable clinical relevance is detected as well. The ADVIA system, which is now unavailable for routine diagnosis, was based on a different technique that

required only low amounts of venom. However, test results in our study were not superior to other techniques of IgE determination.

Although there is considerable progress in the development of diagnostic tools, such as the BAT and CRD, it is still not possible to differentiate between AS, LLRs, and SSRs.

There is a general opinion that sIgE levels to injected venom might be low or might not even be demonstrable in the first few days after a sting.[2] It has also been speculated that low or undetectable IgE levels were due to consumption of circulating IgE antibodies.[20] Therefore we monitored the course of sIgE after the sting closely. First, we tested whether sIgE was "consumed" during the sting. Indeed, there was a statistically significant but, in terms of kilounits per liter, minimal decrease in sIgE levels 3 hours after the sting. One could imagine that free circulating sIgE binds to the delivered venom, resulting in a decrease in sIgE levels. If high-affinity sIgE is available, it should be bound to the allergen regardless of the occurrence of an LLR or SSR or no reaction. However, this effect is minimal at the decimal level, and it ranged, depending on the venom, from −0.08 to −0.13 kU/L. Therefore none of the results in these reactors and nonreactors turned negative after the sting. Generally, guidelines recommend sIgE determination and skin testing not earlier than 2 weeks after the sting because sIgE levels could be decreased or even undetectable.[2,21] However, there is only weak evidence in the literature for this recommendation. Considerations on "IgE consumption" rely on older studies, which showed that sIgE levels turned positive[22] or increased markedly 4 weeks after the sting.[23] It appears from our study that it is not a matter of consumption but a booster effect caused by the sting. Already 1 week after the sting, sIgE levels to wasp venom increased 2.2-fold, and those who were stung by a bee had 2.7-fold increased sIgE levels to bee venom; this is in good agreement with previous studies.[22,23] Generally, sIgE levels decrease in the long term after a sting in allergic patients with[24] or without[25] venom immunotherapy. In our study we observed a decrease to baseline levels within 1 year. Almost identical sIgE kinetics were observed by Golden et al[26] in patients who received immunotherapy. After stopping treatment, patients were challenged annually or biannually over 5 years. They consistently observed an increase in sIgE levels 1 month after each sting, followed by a decrease to the baseline within 1 or 2 years. Importantly, this increase was not associated with therapy failure. To check the clinical relevance of boosted sIgE levels caused by the first sting, we randomly selected subjects for a second challenge. Interestingly, some initially negative IDT and BAT results turned positive after the first sting. However, no subject had an SSR caused by the second sting, and the frequency of LLRs was similar compared with the initial sting. This confirms again that current diagnostic tools cannot predict the occurrence of an LLR or SSR. One limitation of these results might be that the rechallenges were performed 1 year after the first challenge when IgE levels decreased to baseline levels again. We cannot exclude a higher number of SSRs if rechallenges had been performed earlier, such as within 4 weeks, when IgE levels had reached maximum concentrations.

To conclude, sensitization to Hymenoptera venoms is common in the general population. Compared with the sensitization rate, systemic reactions are rare, but the risk for LLRs is markedly increased. This is of particular importance for the interpretation of results of multiplex systems, in which multiple allergen components can be tested with small amounts of serum at the same time and positive results to Hymenoptera venoms are often incidentally observed. Therefore there is no action needed in sensitized subjects who have never experienced allergic symptoms after a sting. There are also 2 important clinical implications regarding the time course of sIgE. First, the increase in sIgE levels after a sting does not indicate conversion into clinically relevant hypersensitivity and is reversible after 1 year. Second, according to our data, sIgE determination can already be done immediately after the sting.

We thank Karin Laipold for her skilled technical assistance.

Key messages

- **Sensitization to Hymenoptera venoms is common in the general population, but SSRs are rare.**
- **The increase in sIgE levels after a sting is not an indicator for conversion into a clinically relevant hypersensitivity.**
- **Currently available tests are not able to distinguish between AS, LLRs, and SSRs.**

REFERENCES

1. Bilo MB, Bonifazi F. The natural history and epidemiology of insect venom allergy: clinical implications. Clin Exp Allergy 2009;39:1467-76.
2. Bilo BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN. Diagnosis of Hymenoptera venom allergy. Allergy 2005;60:1339-49.
3. Bokanovic D, Aberer W, Griesbacher A, Sturm GJ. Prevalence of Hymenoptera venom allergy and poor adherence to immunotherapy in Austria. Allergy 2011; 66:1395-6.
4. Schafer T, Przybilla B. IgE antibodies to Hymenoptera venoms in the serum are common in the general population and are related to indications of atopy. Allergy 1996;51:372-7.
5. Sturm GJ, Schuster C, Kranzelbinder B, Wiednig M, Groselj-Strele A, Aberer W. Asymptomatic sensitization to Hymenoptera venom is related to total immunoglobulin E levels. Int Arch Allergy Immunol 2008;148:261-4.
6. Sturm GJ, Jin C, Kranzelbinder B, Hemmer W, Sturm EM, Griesbacher A, et al. Inconsistent results of diagnostic tools hamper the differentiation between bee and vespid venom allergy. PLoS One 2011;6:e20842.
7. Hemmer W, Focke M, Kolarich D, Wilson IB, Altmann F, Wohrl S, et al. Antibody binding to venom carbohydrates is a frequent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy. J Allergy Clin Immunol 2001;108:1045-52.
8. Sturm GJ, Heinemann A, Schuster C, Wiednig M, Groselj-Strele A, Sturm EM, et al. Influence of total IgE levels on the severity of sting reactions in Hymenoptera venom allergy. Allergy 2007;62:884-9.
9. Sturm GJ, Bohm E, Trummer M, Weiglhofer I, Heinemann A, Aberer W. The CD63 basophil activation test in Hymenoptera venom allergy: a prospective study. Allergy 2004;59:1110-7.
10. Sturm GJ, Kranzelbinder B, Sturm EM, Heinemann A, Groselj-Strele A, Aberer W. The basophil activation test in the diagnosis of allergy: technical issues and critical factors. Allergy 2009;64:1319-26.
11. Hofmann SC, Pfender N, Weckesser S, Huss-Marp J, Jakob T. Added value of IgE detection to rApi m 1 and rVes v 5 in patients with Hymenoptera venom allergy. J Allergy Clin Immunol 2011;127:265-7.
12. Korosec P, Valenta R, Mittermann I, Celesnik N, Erzen R, Zidarn M, et al. Low sensitivity of commercially available rApi m 1 for diagnosis of honeybee venom allergy. J Allergy Clin Immunol 2011;128:671-3.
13. Sturm GJ, Hemmer W, Hawranek T, Lang R, Ollert M, Spillner E, et al. Detection of IgE to recombinant Api m 1 and rVes v 5 is valuable but not sufficient to distinguish bee from wasp venom allergy. J Allergy Clin Immunol 2011;128:247-8; author reply 248.
14. Sturm GJ, Bilo MB, Bonadonna P, Hemmer W, Caruso B, Bokanovic D, et al. Ves v 5 can establish the diagnosis in patients without detectable specific IgE to wasp venom and a possible north-south difference in Api m 1 sensitization in Europe. J Allergy Clin Immunol 2012;130:817; author reply 818-9.
15. Muller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G. Hymenoptera venom allergy: analysis of double positivity to honey bee and *Vespula* venom

by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v5. Allergy 2009;64:543-8.
16. Blaauw PJ, Smithuis OL, Elbers AR. The value of an in-hospital insect sting challenge as a criterion for application or omission of venom immunotherapy. J Allergy Clin Immunol 1996;98:39-47.
17. Parker JL, Santrach PJ, Dahlberg MJ, Yunginger JW. Evaluation of Hymenoptera-sting sensitivity with deliberate sting challenges: inadequacy of present diagnostic methods. J Allergy Clin Immunol 1982;69:200-7.
18. van der Linden PW, Hack CE, Struyvenberg A, van der Zwan JK. Insect-sting challenge in 324 subjects with a previous anaphylactic reaction: current criteria for insect-venom hypersensitivity do not predict the occurrence and the severity of anaphylaxis. J Allergy Clin Immunol 1994;94:151-9.
19. Wright DN, Lockey RF. Local reactions to stinging insects (Hymenoptera). Allergy Proc 1990;11:23-8.
20. Przybilla B, Ring J, Wielgosch J. Der Basophilen-Histamin-Freisetzungstest als diagnostische Methode bei Hymenopterengift-Allergie. Hautarzt 1988;39:662-70.
21. Przybilla B, Rueff F, Walker A, Räwer H, Aberer W, Bauer C, et al. Diagnose und Therapie der Bienen- und Wespengiftallergie. Allergo J 2011;20:318-39.
22. Rieger-Ziegler V, Rieger E, Kranke B, Aberer W. Hymenoptera venom allergy: time course of specific IgE concentrations during the first weeks after a sting. Int Arch Allergy Immunol 1999;120:166-8.
23. Heinig JH, Engel T, Weeke ER. Allergy to venom from bee or wasp: the relation between clinical and immunological reactions to insect stings. Clin Allergy 1988; 18:71-8.
24. Kemeny DM, Lessof MH, Patel S, Youlten LJ, Williams A, Lambourn E. IgG and IgE antibodies after immunotherapy with bee and wasp venom. Int Arch Allergy Appl Immunol 1989;88:247-9.
25. Mosbech H, Christensen J, Dirksen A, Soborg M. Insect allergy. Predictive value of diagnostic tests: a three-year follow-up study. Clin Allergy 1986;16:433-40.
26. Golden DB, Kwiterovich KA, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Discontinuing venom immunotherapy: outcome after five years. J Allergy Clin Immunol 1996;97:579-87.

TABLE E1. Inclusion and exclusion criteria

Inclusion criteria
Legally competent male and female patients age 18-65 y
Detectable sIgE (>0.35 kU/L) to bee or wasp venom
No history of an SSR after a Hymenoptera sting
For female patients, effective contraception
Exclusion criteria
Clear history of a systemic anaphylactic reaction after a Hymenoptera sting
Subjects who have received immunotherapy with bee or wasp venom
Subjects with severe chronic illness
Severe asthma: FEV_1 < 80% of predicted value or FEV_1/forced vital capacity ratio < 70%
Severe disorders of the lungs, liver, kidneys, or nervous system
Clear chronic or acute cardiovascular failure
Hypertension and/or severe chronic ischemic heart disease
Patients receiving angiotensin-converting enzyme inhibitor or β-blocker treatment
Severe psychological disorders
For female subjects, pregnancy and breast-feeding